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PHOTOBIOLOGY OF THE SYMBIOTIC SEA ANEMONE, *ANTHOPLEURA ELEGANTISSIMA:* DEFENSES AGAINST PHOTODYNAMIC EFFECTS, AND SEASONAL PHOTOACCLIMATIZATION

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Abstract

The sea anemone *Anthopleura elegantissima,* which contains photosynthetic symbionts (zooxanthellae), responds both biochemically and behaviorally to the combined environmental stresses of exposure to sunlight and photosynthetically generated hyperbaric O_2. Activities of the enzymes superoxide dismutase (SOD) and catalase, which act in concert as defenses against oxygen toxicity, parallel the distribution of chlorophyll. *A. elegantissima* shows a finely controlled contraction behavior which shades the zooxanthellae and reduces O_2 production, but which leaves the body column tissues directly exposed to sunlight. However, the body column contains disproportionately high SOD and catalase activities as defenses against photodynamic damage. This additional role of SOD is demonstrated by shade-adapted aposymbiotic anemones in which SOD and catalase activities increase by 590% and 100% respectively following a 7 day exposure to sunlight. In response to elevated levels of O_2 and sunlight exposure, *A. elegantissima* attaches gravel and other debris to its body surface which serves as a sunscreen that effectively reduces zooxanthella expulsion during exposure to bright sunlight. Finally, anemone chlorophyll content fluctuates on a seasonal basis, varying inversely with mean solar radiation. These seasonal changes are not due to corresponding changes in the number of algal cells, but rather to changes in the chlorophyll content and chlorophyll $a{:}c_2$ ratio of a fairly uniform standing crop of zooxanthellae.

Introduction

Much of the recent interest in plant-animal symbioses has focused on nutritionally beneficial aspects of these associations. Mutually advantageous exchanges of organic and inorganic metabolites between photosynthetic endosymbiont and host animal have been demonstrated (Trench, 1979; Muscatine, 1980; Cook, 1983) and attempts to quantify the contributions made by photosynthetically fixed carbon to the host's metabolic needs have been progressively refined (McCloskey *et al.,* 1978; Muscatine *et al.,* 1981; Muscatine *et al.,* 1984). There are, however, a number of potentially deleterious interactions between the host and symbiont that are unavoidable consequences of the nature of these symbioses, namely the toxicity of molecular oxygen produced as an obligatory by-product of photosynthesis and the requirement for sunlight exposure and the associated potential for photodynamic damage.

Although the molecular bases for oxygen toxicity are not generally agreed upon (Halliwell, 1978, 1982; Fee, 1980; Sullivan *et al.,* 1983), it is evident that because of

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orbital spin restrictions molecular oxygen preferentially undergoes univalent reductions which, directly or indirectly, can generate the superoxide radical (O_2^-), the hydroxyl radical ($OH^\cdot$) and hydrogen peroxide (H_2O_2) (Halliwell, 1979; Fridovich, 1977a, b, 1978; Cadet and Teoule, 1978; Hill, 1978), three of the most reactive chemical species in biological systems. The enzymes superoxide dismutase (SOD) and catalase act in concert to minimize the toxicity of oxygen by removing superoxide radicals and H_2O_2, thereby preventing the formation of hydroxyl radicals (McCord and Fridovich, 1969; Fridovich, 1978; Steinman, 1982; Chapman and Schopf, 1983). In addition to ultraviolet (UV) wavelengths of sunlight below 380 nm, long recognized as damaging to biological materials (Clayton, 1977; Jokiel, 1980; Jokiel and York, 1982), wavelengths of visible light are also responsible for a variety of biologically disruptive reactions that are synergistically mediated by the presence of molecular oxygen (Clayton, 1977). These reactions, dependent on the presence of photosensitizing agents (*e.g.,* flavins, chlorophyll), include single electron transfers capable of generating superoxide radicals, as well as photochemical oxygenations and photoproduction of highly reactive singlet oxygen (Clayton, 1977; Khan, 1978; van Ginkel and Raison, 1980; Cooper and Zika, 1983).

The abundance of photosynthetically generated molecular oxygen and concurrent exposure to sunlight experienced by animals containing symbiotic algae present ideal conditions for biologically damaging interactions between sunlight and oxygen. This may be particularly true for sessile, soft-bodied marine invertebrates that occupy the intertidal region and are directly exposed to sunlight.

The West Coast intertidal sea anemone *Anthopleura elegantissima* (Brandt) harbors intracellular dinoflagellates (zooxanthellae) as endosymbionts and shows biochemical and behavioral adaptations to the environmental stresses of intense irradiance and simultaneous exposure to hyperbaric oxygen. Biochemical adaptations include maintaining levels of SOD and catalase activity in direct proportion to its algal complement (Dykens, 1984) as well as the presence of a UV absorbing pigment in anemones accustomed to sunlight exposure (Shick and Dykens, 1984). In addition to these biochemical defenses, *A. elegantissima* contracts during periods of peak irradiance (Pearse, 1974; Shick and Dykens, 1984) and attaches a cover of shells and other debris to its body column (Hart and Crowe, 1977; Dykens and Shick, 1983), both of which shade the zooxanthellae and reduce exposure to sunlight. However, acute exposure to intense sunlight causes *A. elegantissima* to expel large numbers of intact zooxanthellae (Pearse, 1974), a response also seen in other symbiotic sea anemones (Steele, 1976). Finally, unlike tropical symbiotic species, the temperate *A. elegantissima* experiences varying intensities of solar radiation that fluctuate seasonally by 450% even in central areas of its distribution (Barbour *et al.,* 1973). In this report we examine more fully the localization of anemone SOD and catalase and present evidence of an additional role for these enzymes as defenses against photodynamic damage. We also examine the effectiveness of attached debris as a sunscreen and present data on seasonal variations in numbers of zooxanthellae and chlorophyll content.

Materials and Methods

All specimens of *Anthopleura elegantissima* (Brandt) were collected from the intertidal region of the south jetty at the entrance of Bodega Harbor, California. Anemones were taken from several low intertidal clones at mean lower low water and from high intertidal clones 1.3 m above that level. Aposymbiotic anemones totally lacking symbiotic algae were collected from constantly shaded crevices along the jetty at a mid-intertidal height. Anemones were returned to Bodega Marine Laboratory

and placed in an outdoor flowing sea water system (14°C) in sunlight (for irradiance data see Shick and Dykens, 1984).

Enzyme determinations and localization

Freshly collected low-intertidal anemones were dissected into the tissues of the tentacles and oral disc, body column, and basal disc which were individually weighed and gently homogenized in sea water (Brinkmann Polytron homogenizer; 10% wt/vol). An aliquot of the crude tissue homogenate was removed for chlorophyll extraction prior to centrifugation at 900 × *g* for 10 min. Both chlorophyll *a* and c_2 were calculated using the equations of Jeffrey and Humphrey (1975) following two 10 h extractions of the crude homogenate in 90% spectroanalyzed acetone in the dark (4°C). No chlorophyll could be detected in the supernatant using this or the more sensitive fluorometric technique of Yentsch and Menzel (1963), indicating that the zooxanthellae had not been disrupted during the homogenization procedure. Superoxide dismutase (SOD) activity of the supernatant, determined by the polarographic technique of Tyler (1975), is expressed in units according to McCord and Fridovich (1969). Catalase activity, expressed in standard Sigma units, was measured using the polarographic procedure of Goldstein (1968). All enzyme assays were performed at 14°C. Soluble protein content of the supernatant was determined using the microbiuret method of Itzhaki and Gill (1964) with bovine serum albumin as the standard.

Enzyme activity in aposymbiotic anemones

Aposymbiotic anemones were collected from several crevices along the jetty and therefore undoubtedly represent multiple clones. Intact anemones were homogenized immediately upon return to the laboratory (No Treatment, Fig. 1A, B) and protein content, SOD, and catalase activities of the supernatant determined as described above. Additional groups of 10 anemones were kept immersed in flowing sea water under the following conditions (Fig. 1A, B): in the dim artificial illumination of the laboratory (maximum irradiance 25 μEinsteins $\cdot$ $m^{-2} \cdot s^{-1}$) (Dim); in dim illumination of the laboratory but continuously exposed to 320 mmHg (0.42 atm) exogenous O_2, a P_{O_2} similar to that measured in tissues of symbiotic anemones in light (Dykens and Shick, 1982) (Dim High O_2); outdoors in full sunlight during the daylight hours (Sun); outdoors in full sunlight but beneath clear sheets of plate glass to block ultraviolet radiation (UV Blocked). After seven days in the above treatments, the anemones were homogenized and chlorophyll extractions performed. Following centrifugation, supernatant protein content and SOD and catalase activities were determined.

Attachment of gravel

Symbiotic anemones from a single high and single low intertidal clone were collected and cleaned of all attached gravel, shell, and other debris using forceps. Groups of 10 anemones were separated immediately into individual aquaria and were kept continuously immersed in running sea water under the following conditions (Fig. 3): outdoors in ambient sunlight (Normal); outdoors in sunlight but beneath plate glass to block ultraviolet wavelengths (UV Blocked); outdoors in sunlight but exposed to 10^{-5} M DCMU (3-3,4-dichlorophenyl)-1,1-dimethylurea), an inhibitor of photosynthesis (DCMU); indoors under the dim illumination of the laboratory (Dim); indoors but continuously exposed to 0.42 atm oxygen (Dim High O_2). *A. elegantissima* preferentially attaches gravel of 1–1.5 mm diameter to the verrucae of its body column (Hart and Crowe, 1977; pers. obs.), and gravel of this size, sieved from the

beach at Bodega Marine Laboratory, was provided to the groups of anemones in a ratio of 5 g gravel to 1 g anemone wet wt. Most (95–99%) of the gravel remained unattached at the end of the experimental period indicating that it had been supplied in excess. After 12 h in the above treatments, the anemones were removed and the attached gravel picked off with forceps, allowed to dry in air for 48 h, and weighed. The anemones were weighed, homogenized, and their chlorophyll contents determined.

Expulsion of zooxanthellae

Anemones from a single mid-intertidal clone from an exposed and brightly sunlit boulder were collected and returned to the laboratory. Fifteen individuals were immediately cleaned of attached debris, homogenized, and their chlorophyll contents determined (No Treatment, Fig. 4). Three other groups of 15 clonemates each were kept continuously immersed outdoors in flowing sea water (Fig. 4). Two of these groups were allowed to retain their natural cover of shell bits and gravel (Debris Attached) and one of these was continuously exposed to 0.42 atm exogenous O_2 (Debris Attached High O_2). The third group was cleaned of all attached debris before being placed outdoors (Debris Removed). After 12 h, anemones from all treatments were cleaned of debris, homogenized, and their chlorophyll contents determined.

Individuals were also collected from a second mid-intertidal clone from a similarly sunny habitat. Chlorophyll content was determined for these field-fresh anemones (No Treatment Clone 2) and after 12 h exposure to 0.42 atm O_2 in the dim illumination of the laboratory (Dim High O_2 Clone 2) (Fig. 4).

Seasonal zooxanthella counts and chlorophyll determinations

An aliquot of the crude homogenate was diluted in an appropriate volume of Moore's calcium-free sea water (Cavanaugh, 1975) plus 0.5 m*M* EDTA. Zooxanthellae were counted using a Neubauer hemocytometer and a Zeiss transmission fluorescence microscope. After digestion in 5% NaOH (12 h, 25°C), total protein content of the homogenate was determined by the dye binding technique of Bradford (1976) using bovine albumin as the standard.

Except for 36 individuals in the month of June (Fig. 5) which were analyzed immediately after collection, all seasonal chlorophyll data are for anemones collected from the jetty and shipped by air to our laboratory in Maine where they were maintained at the habitat temperature in aerated sea water under dim illumination for a maximum of 48 h before homogenization.

Results

Regional enzyme activity

The distribution of chlorophyll among several regions in the anemone supports the subjective impression that most of the endosymbiotic algae are found within the tentacles and oral disc, while fewer are located in the endoderm of the body column and none in the basal disc (Table I). The activities of the protective enzymes SOD and catalase parallel this chlorophyll distribution: the highest SOD and catalase activities are found in the alga-rich tentacles and oral disc, while intermediate levels are found in the body column and the lowest activities in the alga-free basal disc (Table I).

TABLE 1

Distribution of total chlorophyll ($a + c_2$), *superoxide dismutase (SOD) and catalase activities among body regions of the sea anemone* Anthopleura elegantissima

Region	Chlorophyll[1]	SOD[2]	Catalase[2]
Tentacles and oral disc	20.18 ± 2.27	58.87 ± 15.63	150.97 ± 22.13
Body column	1.53 ± 0.61	21.75 ± 7.18	47.07 ± 6.54
Basal disc	0.00	8.73 ± 3.13	34.02 ± 7.42

[1] $\mu g \cdot mg^{-1}$ supernatant protein.
[2] Units $\cdot mg^{-1}$ supernatant protein.
Values given are means ± S.D., n = 5.

Enzyme activities in aposymbiotic anemones

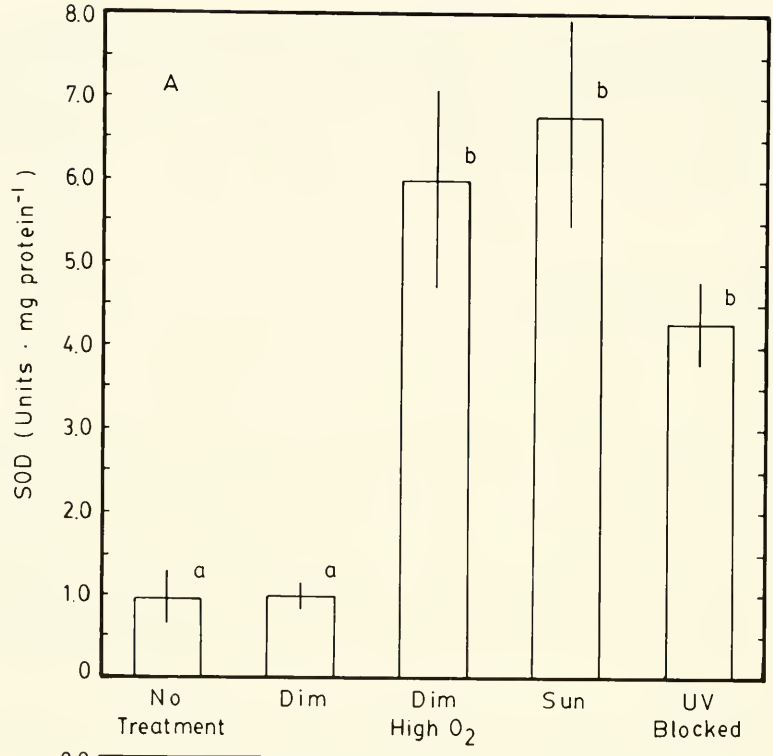
Corroborating previous findings (Dykens and Shick, 1982; Dykens, 1984), SOD activity is low in freshly collected aposymbiotic anemones from shaded habitats (No Treatment, Fig. 1A) and is unchanged after one week maintenance in the dim illumination of the laboratory (Dim, Fig. 1A). However, shade-adapted aposymbiotic anemones exposed to sunlight for one week show a 590% increase in SOD activity ($P < 0.001$) (Sun, Fig. 1A) and a 340% increase ($P < 0.001$) even when protected from UV wavelengths (UV Blocked, Fig. 1A). Similar large increases in SOD activity are induced by exposure to elevated levels of oxygen while in dim illumination (Dim High O_2, Fig. 1A).

Catalase activity of shade-adapted aposymbiotic anemones shows trends similar to SOD activity in that anemones both freshly collected (No Treatment, Fig. 1B) and after one week of laboratory maintenance (Dim, Fig. 1B) have low enzyme activities which can be increased by exposure to sunlight (either with or without UV) or to elevated oxygen. The latter is the most effective (Fig. 1B). No chlorophyll could be detected in any of these anemones after the one week experimental period, indicating that they had not acquired zooxanthellae from other symbiotic anemones in the same flowing sea water system.

It should be noted that the SOD and catalase specific activities reported here are expressed per unit soluble protein in the supernatant and are not directly comparable to our previous data (Dykens and Shick, 1982; Dykens, 1984) where activities are expressed per unit total protein in the anemones.

Attachment of gravel

Continuously immersed high- and low-shore symbiotic anemones, cleaned of debris and placed in sunlight, attach gravel to their body surface in direct proportion to the amount of chlorophyll they contain (Fig. 2 presents data for Normal treatments shown in Fig. 3). Moreover, high-shore anemones (closed circles, Fig. 2; Fig. 3) always attach more gravel per unit chlorophyll than do low-shore anemones (open circles, Fig. 2; Fig. 3) ($F = 65.80$, $n = 100$, $P < 0.0005$) and this is true regardless of experimental treatment (Fig. 3). Although analysis of variance does not reveal significant differences among the four experimental treatment groups of high-shore anemones, all experimental groups of high-shore anemones attach significantly ($P < 0.05$) less gravel than do sunlight-exposed controls (Normal, Fig. 3). In low-shore anemones the extent of gravel attachment can be experimentally manipulated: low-shore anemones in all four treatment groups attach significantly less gravel than


A
SOD (Units · mg protein^{-1})
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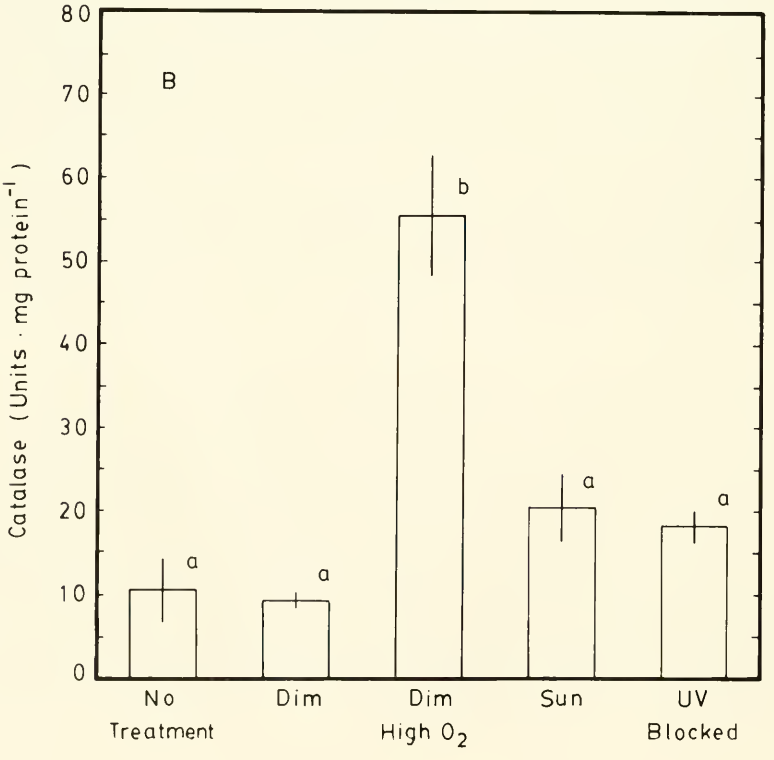
B
Catalase (Units · mg protein^{-1})
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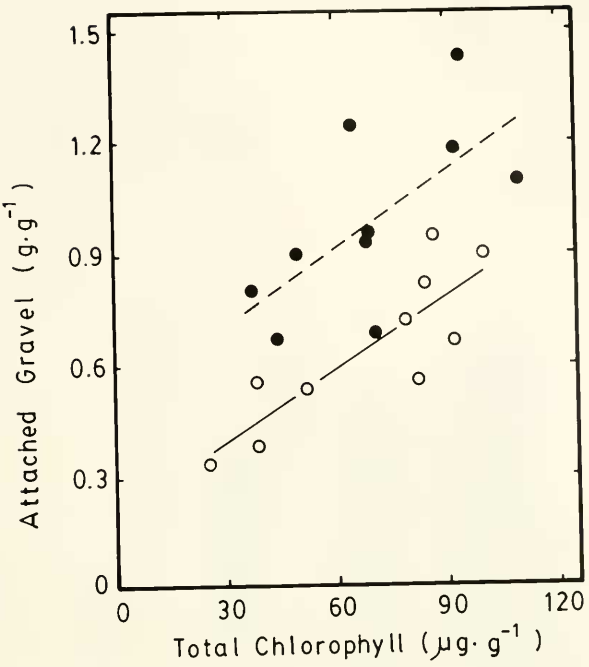

FIGURE 2. Relationship between total chlorophyll ($a + c_2$) content ($\mu g \cdot g^{-1}$ anemone wet wt) and weight of gravel attached to body surface ($g \cdot g^{-1}$ anemone wet wt) by continuously immersed, high-shore (closed circles; $Y = 7.0 \times 10^{-3} X + 0.489$, $r = 0.648$, $P < 0.025$) and low-shore (open circles; $Y = 6.6 \times 10^{-3} X + 0.188$, $r = 0.852$, $P < 0.001$) *A. elegantissima* after 12 h exposure to sunlight.

normal sunlight-exposed controls and this amount is decreased further by inhibiting the photosynthetic production of oxygen either chemically (DCMU) or by lowered irradiance (Dim). Conversely, low-shore anemones in dim illumination, but exposed to elevated exogenous oxygen (Dim High O_2, Fig. 3) attach as much gravel as anemones undergoing photosynthesis in sunlight without UV. Both low- and high-shore anemones exposed to full sunlight, but protected from UV (UV Blocked, Fig. 3), attach significantly ($P < 0.05$) less gravel than clonemates exposed to sunlight containing UV wavelengths (Normal, Fig. 3).

Gravel as sunscreen

The effectiveness of attached debris as a sunscreen is shown by the zooxanthella expulsion data presented in Figure 4. When allowed to retain their attached debris (Debris Attached), anemones from this mid-intertidal clone lose no significant amount of chlorophyll after 12 h of sunlight exposure compared to field-fresh clonemates (No Treatment). During the same time period, however, clonemates in sunlight, but de-

FIGURE 1. A. Superoxide dismutase (SOD), and B. catalase activities in freshly collected, shade-adapted aposymbiotic *A. elegantissima* (No Treatment) and after one week exposure to dim illumination in the laboratory (Dim), full sunlight (Sun), sunlight without UV (UV Blocked), and dim illumination but constant exposure to 320 mmHg O_2 (Dim High O_2). Mean ± S.D., n = 10. Means not significantly different at $P < 0.001$ (one way analysis of variance; Student-Newman-Keuls multiple comparison test) share superscripts.

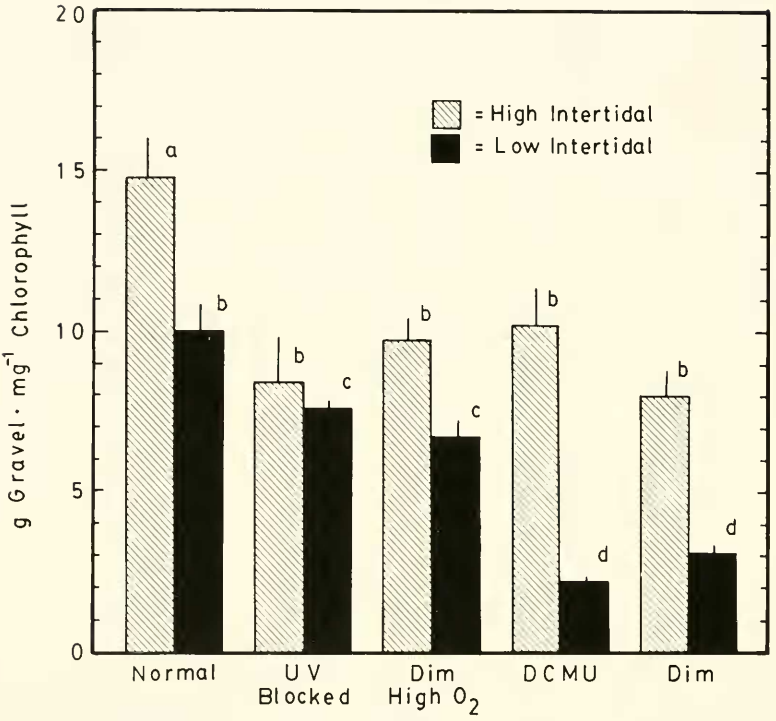

FIGURE 3. Gravel attachment by high-shore (hashmarked) and low-shore (solid bars) symbiotic *A. elegantissima* after 12 h exposure to gravel under various conditions (see text for full explanation of treatments). Mean ± S.D., n = 10. Means not significantly different at $P < 0.001$ (two-way analysis of variance; Student-Newman-Keuls) share superscripts.

prived of attached cover (Debris Removed), lose significant ($P < 0.01$) amounts of chlorophyll. This loss of zooxanthellae also can be induced by continuously exposing sunlit anemones with debris attached to 320 mmHg oxygen (Debris Attached High O_2, Fig. 4). To test whether elevated O_2 alone is the proximal cause of the observed expulsion of zooxanthellae, anemones from a second clone from a habitat similar to that of the first were continuously exposed to 0.42 atm O_2 under dim illumination for 12 h. These anemones (Dim High O_2 Clone 2, Fig. 4) lose no significant amount of chlorophyll compared to freshly collected clonemates (No Treatment Clone 2, Fig. 4).

Seasonal changes in numbers of zooxanthellae and chlorophyll content

All experiments reported here and previously (Shick, 1981; Dykens, 1984; Shick and Dykens, 1984) were conducted in June of 1980, 1982, and 1983, a month chosen to minimize air/sea water temperature differences (Barbour *et al.,* 1973). This also coincides with the period of least difference in chlorophyll content between high- and low-shore anemones (Fig. 5A).

There is a seasonal cycle in anemone chlorophyll content that varies inversely with mean daily solar radiation (Fig. 5A). Seasonal differences are more pronounced in low-shore anemones, but both low- and high-shore individuals contain more total chlorophyll during the winter months, when irradiance is at its lowest, and less chlo-

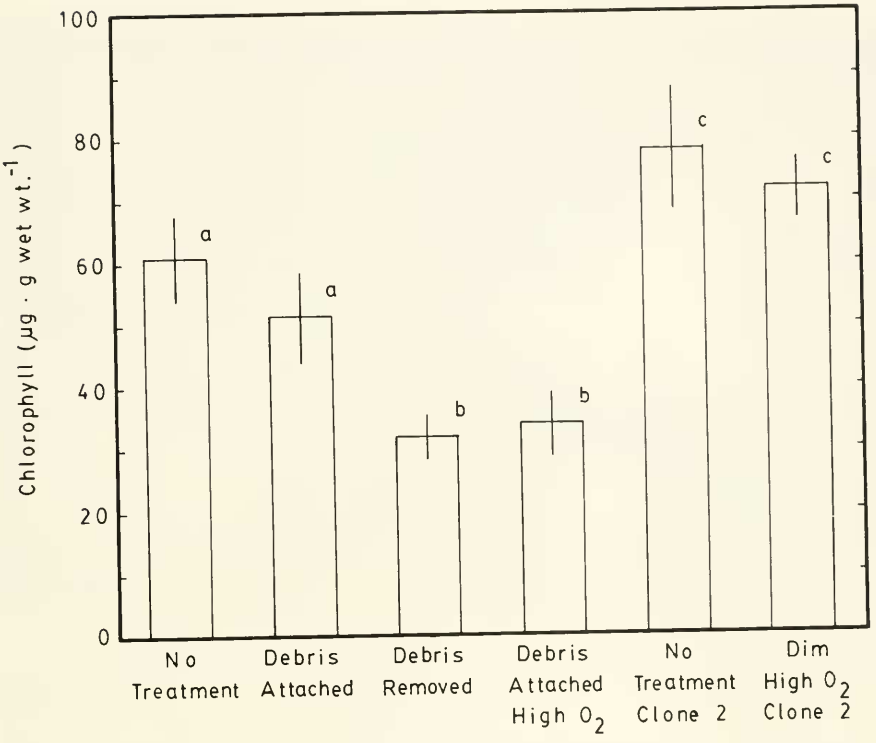

FIGURE 4. Effect of debris attached to the body surface on expulsion of zooxanthellae from *A. elegantissima* after 12 h under various conditions (see text for explanation of treatments). Mean ± S.D., n = 15. Means not significantly different at $P < 0.001$ (one way analysis of variance; Student-Newman-Keuls) share superscripts.

rophyll during the brighter period of June to September (Fig. 5A). Although the total chlorophyll content of low-shore anemones varies about 5-fold between September and December, the number of algal cells in *A. elegantissima* remains fairly constant throughout the year (Fig. 5B). Both chlorophylls *a* and c_2 increase as average daily solar radiation decreases, but chlorophyll c_2 increases disproportionately which reduces the chlorophyll $a{:}c_2$ ratio during the winter months (Fig. 5B).

DISCUSSION

The sea anemone *Anthopleura elegantissima* shows biochemical and behavioral adaptations that allow it to exploit the nutritional benefits provided by its photosynthetic endosymbionts while minimizing the potential of oxygen toxicity and photodynamic damage. As a long-term adaptation to avoid the cytotoxicity of molecular oxygen, this anemone maintains SOD and catalase activity in direct proportion to its chlorophyll content, an estimator of potential oxygen production (Dykens, 1984). Moreover, the localization within the anemone of these enzymes which act to minimize oxygen toxicity by maintaining low cellular levels of O_2^- and H_2O_2 (Fridovich, 1978), directly parallels regional chlorophyll distributions (Table I). Most of the chlorophyll, and consequently most of the oxygen production, is found in the tentacles and oral disc which also contain the highest activities of the two protective enzymes (Table I).

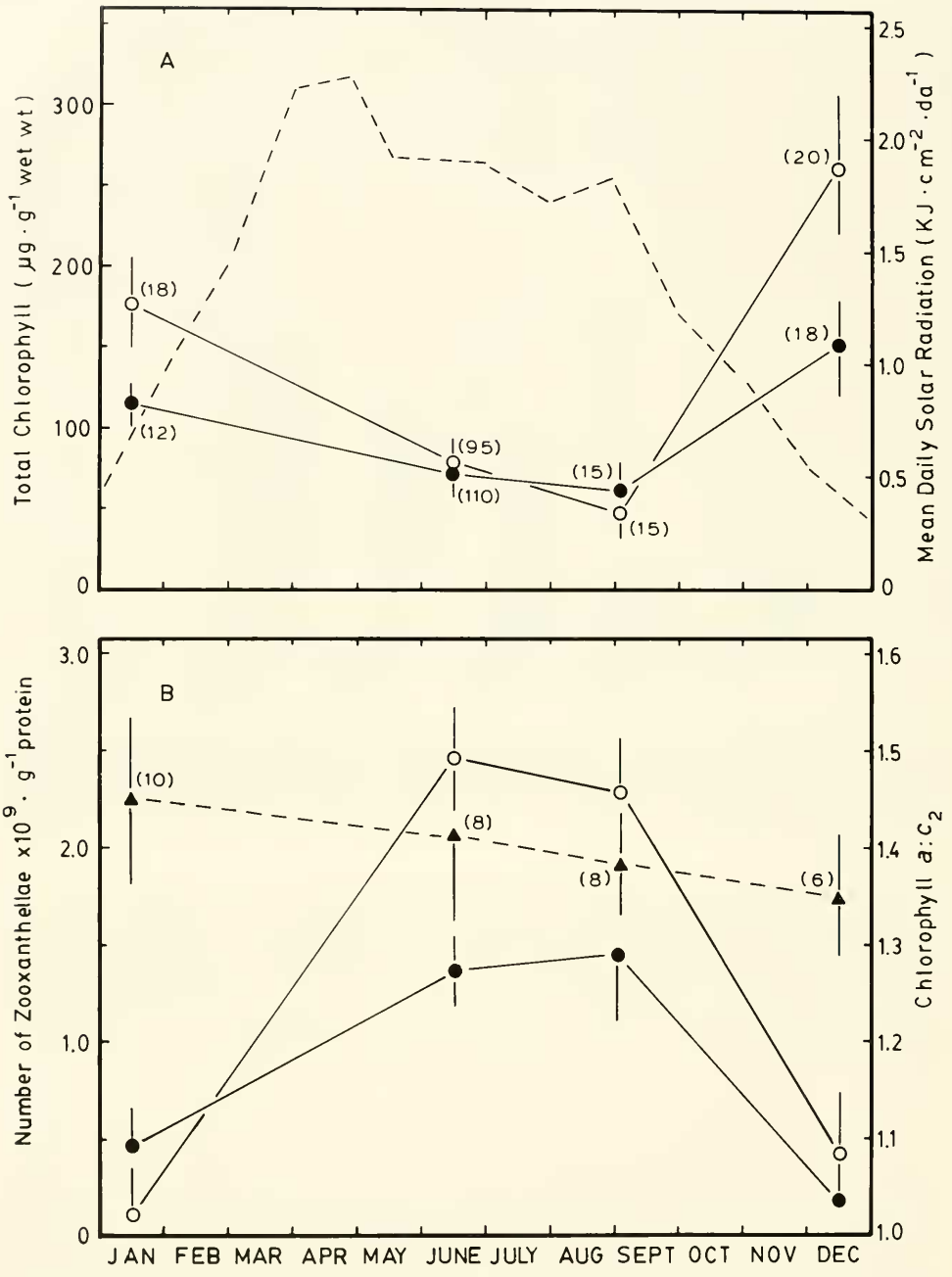

FIGURE 5. A. Seasonal fluctuation in total chlorophyll ($a + c_2$) content ($\mu g \cdot g^{-1}$ anemone wet wt) of high-shore (closed circles) and low-shore (open circles) *A. elegantissima* (Mean ± S.D., n indicated) as well as average daily solar radiation at the collection site (dashed line). Irradiance data redrawn from Barbour *et al.* (1973) with permission. The plateau in mean daily solar radiation seen from mid-May to September is due to prevalent fog in June, July, and August, which is normal for this period (Barbour *et al.*, 1973). B. Seasonal variation in number of zooxanthellae (number algal cells $\cdot g^{-1}$ total protein) isolated from low-shore *A. elegantissima* (solid triangles) (Mean ± S.E., n indicated) and chlorophyll $a:c_2$ ratios for high-shore (closed circles) and low-shore (open circles) anemones. (Mean ± S.E., n same as for total chlorophyll in Fig. 5A.)

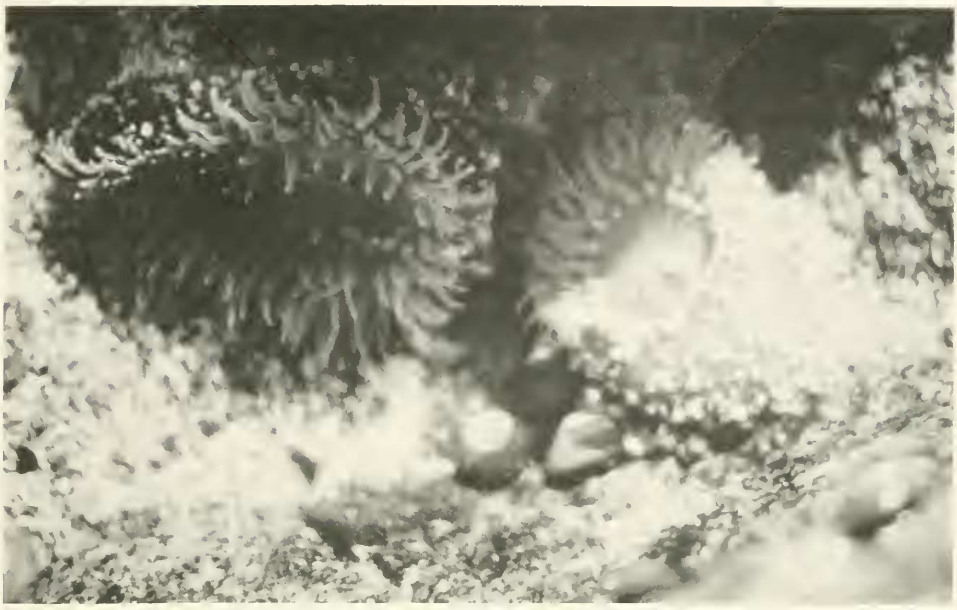

FIGURE 6. Localized retraction of tentacles and contraction of radial muscles in the oral disc by immersed *Anthopleura xanthogrammica* in response to bright sunlight (550 μEinsteins $\cdot m^{-2} \cdot s^{-1}$). Note that the shaded portion of the anemone remains expanded, as does the entirely shaded anemone at left. Also note the gravel attached to the verrucae of the body column.

In addition to these enzymatic defenses, *A. elegantissima* shows behavioral responses that reduce exposure to intense sunlight. During intervals of high irradiance, this anemone retracts its alga-containing tentacles and contracts its marginal sphincter which constricts the oral disc (Pearse, 1974; Shick and Dykens, 1984). This behavior, which shades the majority of the zooxanthellae thereby reducing the production of oxygen, occurs in response to elevated levels of oxygen and to sunlight exposure *per se* (Shick and Dykens, 1984). It is a finely modulated behavior in that immersed anemones partially exposed to sunlight retract only those tentacles and contract that area of the oral disc that are directly illuminated, behaviors perhaps mediated by local electrical conduction systems (Marks, 1976; McFarlane, 1984) or by the effects of light on the muscles themselves (North and Pantin, 1958), while the shaded portion of the anemone remains expanded (shown in the congeneric *A. xanthogrammica* in Fig. 6).

Reduced photosynthesis ($P_z$net) during periods of peak irradiance, due to contraction in *A. elegantissima,* is also seen in symbiotic corals (Porter, 1980) and tridacnid clams (Mangum and Johansen, 1982) and will reduce estimates of the contribution made by zooxanthellae to animal respiration (CZAR). The impact of reduced $P_z$net on CZAR is only partially offset by concurrent reductions in animal respiration while contracted (Shick and Dykens, 1984). Both of these factors reinforce the need for long-term monitoring of O_2 flux under natural intensities of irradiance if any appraisal of the nutritional status of symbiotic associations is to be environmentally realistic.

Even when *A. elegantissima* is fully contracted, the superficial tissues of the body column remain exposed to direct sunlight and possible photodynamic damage. Moreover, the zooxanthellae within the column are also exposed to sunlight and the sessile anemone has no means, other than attaching a shading sunscreen (see below), to modulate O_2 production by these algae. However, there is five times more SOD

activity and four times more catalase activity in the body column of *A. elegantissima* than predicted on the basis of the enzyme to chlorophyll ratios observed in the tentacles and oral disc. These inordinately high levels of enzyme activities may reflect an additional need for cellular defenses against photogenerated oxygen radicals above those required by the amount of chlorophyll (and subsequent O_2 production) in this region.

Supporting the suggestion that SOD and catalase are defenses against photodynamic damage mediated by oxygen radicals is the finding that aposymbiotic anemones, previously unexposed to direct sunlight, show a 590% increase in SOD activity and a 100% increase in catalase activity following a one week exposure to sunlight (Fig. 1). In fact, sunlight exposure is as effective as exposure to hyperbaric oxygen in inducing SOD activity in aposymbiotic anemones (Fig. 1A). Less energetic wavelengths of visible light are responsible for most of the increase in SOD activity: anemones exposed to sunlight, but protected from UV, possess fully two-thirds the SOD activity of anemones exposed to sunlight containing UV.

Catalase activity shows a qualitatively similar, if somewhat less spectacular, pattern. The trend towards increased catalase activity seen in sun-exposed anemones is also seen in anemones protected from UV (Fig. 1B), again indicating that these enzymic responses are due primarily to visible wavelengths of light. It may be that the catalytic capacity of pre-existing catalase is nearly adequate to cope with the increased production of H_2O_2 due to the sun-induced increase in SOD activity as well as the normal production of H_2O_2 from other cellular processes (Dykens, 1984). This is not the case, however, following chronic exposure to hyperbaric oxygen: anemones indoors but exposed to 0.42 atm O_2 show a 5-fold increase in catalase activity. Perhaps exposure to elevated oxygen increases H_2O_2 production from metabolic processes that are independent of SOD activity such as increases in glucose oxidase activity and oxidations involving reduced flavoproteins.

In addition to the inordinately robust enzymic defences in the column tissues, *A. elegantissima* attaches gravel, calcareous fragments, and pieces of macroalgae to the verrucae of the body column. This attached debris not only effectively decreases desiccation during aerial exposure (Hart and Crowe, 1977), but also serves as a sunscreen that shades the body column and reduces O_2 production by algae in the column as well as the potential for photodynamic damage. A preliminary analysis of 20 anemones from a single clone, 10 from the sunny side and 10 from the shady side of a mid-intertidal boulder, indicated that the more brightly illuminated anemones attached more debris (0.80 mg per mg anemone dry wt $\pm$ 0.16 S.D., *versus* 0.43 mg per mg anemone dry wt $\pm$ 0.20; $t = 4.51$, $P < 0.001$). Since these anemones were routinely exposed to air, it could be argued that the principal role of the light-colored gravel was to reflect sunlight and to maintain a low body temperature (which would also reduce evaporation) in the exposed anemones. However, continuously immersed anemones from both low- and high-shore clones attach gravel in direct proportion to the amount of chlorophyll they contain (Fig. 2). In fact, for low-shore anemones which experience less desiccation, photosynthetic production of oxygen appears to be the proximal determinant of gravel attachment, although exposure to UV also plays a role (Fig. 3). High-shore anemones, however, consistently attach a great deal of gravel regardless of experimental treatment. It may be that in these anemones, which are conditioned to aerial exposure, the requirement for gravel as a defense against desiccation is paramount, so that they attach it whenever it is available. Even these high-shore anemones, however, attach less gravel when protected from UV.

The attached debris is an effective sunscreen. Anemones allowed to retain their cover also retain their zooxanthellae when exposed to full sunlight, while clonemates deprived of debris expel half their algal complement under the same conditions

(Fig. 4). This loss of zooxanthellae appears to involve an interaction between irradiance and high intracellular O_2 levels: sun-exposed anemones with debris attached expel their algae only when exposed to hyperbaric O_2, while anemones exposed to identical oxygen levels, but kept under low illumination, retain their algae.

The seasonal fluctuations in anemone chlorophyll content, which varies inversely with average solar radiation, cannot be attributed to corresponding changes in the number of zooxanthellae, but are due to changes in the chlorophyll content of a fairly constant standing crop of algal cells (Fig. 5A, B). These seasonal fluctuations are more pronounced in low-shore compared to high-shore anemones, perhaps due to the compounding factor of seasonally changing sea water turbidity and corresponding attenuation of sunlight reaching more deeply submerged low-shore clones. Also, high-shore anemones are directly exposed to sunlight at low tide, which may limit the amount of chlorophyll they can safely contain. Regardless, algae from both high- and low-shore anemones contain more chlorophyll during the winter months (Fig. 5A), probably not as a response to lower sea water temperatures which vary annually by only 6°C (Barbour *et al.,* 1973), but as an adaptation which enhances use of the seasonally reduced solar flux. Although both chlorophylls *a* and c_2 increase as average daily irradiance decreases, disproportionate increases in chlorophyll c_2 relative to chlorophyll *a* (Fig. 5B) imply that photoacclimatization of zooxanthellae in *A. elegantissima* occurs primarily *via* proliferation of accessory antenna pigments within existing photosynthetic units (PSUs) (Prézelin, 1981). There are no O_2 flux data available directly comparing maximum rates of photosynthesis of winter and summer acclimatized anemones. If found to exist, seasonally similar levels of maximum O_2 production would support the proposition that algal photoacclimatization is essentially complete and that it occurs *via* increases in the size, not in the number, of PSUs as is the case for zooxanthellae from other (Dustan, 1982; Chang *et al.,* 1983), but not all (Chang *et al.,* 1983), symbiotic associations.

Seasonally altering chlorophyll concentration represents a means whereby the zooxanthellae can maximize photosynthetic potential in a changing photic environment on a long-term basis. However, maintaining chlorophyll levels sufficient to maximize photosynthetic capacity under average light conditions may expose the host anemone during brief periods of peak irradiance to levels of hyperbaric oxygen and photodynamic interactions that are capable of overwhelming enzymic defenses. *A. elegantissima* responds to these short-term increases in photon flux behaviorally, by contracting and attaching an effective sunscreen of debris to its exposed body surface.

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